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14. ABSTRACT

In this proposal, we will identify and clone a protein that can be used to generate infection-specific gene therapy vector. We expect that using this protein to modify various gene therapy vectors, we can specifically deliver cytotoxic genes into prostate cancer cells using systemic treatment, and eventually eradicate metastatic prostate cancer cells in patients. During the third year, we inserted the sequences that encode these two peptides to modify lentiviral vector envelop gene and used one of the modified membrane proteins to target prostate cells. The viral vector that have peptide YDSVLALSAALQATR (P2) modified gp41 envelope protein was used to infect LNCaP cells in 24-well plates. The control vector that does not have gp41-P2 envelope protein on viral surface was also used to infect LNCaP cells as the control. Our results demonstrated that with the gp41-P2 envelope protein on the surface of the viral vector, lentiviral vectors increase infectivity by 30% to 70% .

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Table of Contents

Title: Identification of a Protein for Prostate-Specific Infection
PI: Shen Pang
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Introduction:

Using gene therapy vectors to eradicate prostate cells is extensively studied. It is expected that if we can use a target-specific gene therapy to deliver cytotoxic genes into metastatic prostate cancer cells in patients, we can eradicate these malignant cells and cure the patients. To find an approach to generate infection-specific vectors is critical for target-specific cancer cell eradication. Because viral vectors infect cells by the binding of their surface proteins to their receptors on the target cells, it is expected that infection-specific vectors can be generated by modifying their surface proteins with proteins that can specifically bind to target cells. In our Preliminary Studies, we found that the lentiviral vector (an HIV-based retroviral vector) generated from a cell line derived from human oral tissue (HOT) can specifically infect LNCaP prostate cancer cells. Virus generated from other cell lines has much lower infectivity to LNCaP cells (200 to 1200-fold lower). These results strongly suggest that the HOT cells express a very specific membrane protein that can be picked by lentiviral vectors to modify their envelope. With such protein on its envelope, the viral vectors are able to specifically infect LNCaP cells.

If this is the case, we expect that a protein for generating infection-specific vector can be obtained by screen the cDNA library of the HOT cell line.

Body:

The approved SOW is listed below.

Task 1. To prepare cDNA library in eukaryotic gene expression vectors (months 1-8).

Task 2. To perform first-round screening to identify the cDNA clone groups that contain the cDNA clones encoding the protein responsible for tissue-specific infection. (months 9-20)

Task 3. To perform the second round of screening to identify the individual clones that contain the cDNA encoding the protein responsible for tissue-specific infection (months 21-24)

Task 4. To sequence the identified gene (months 25-26)

Task 5. To use sequence analysis to characterize the identified gene (months 27-28)

Task 6. To generate deletions of the identified gene and to use them to confirm the functional domains of the identified gene (months 31-36)

To follow the schedule, we should complete all the Tasks. We have identified two peptides that can significantly increase tissue-specific gene delivery as we reported in the annual report of last year (Task 1-4). In the past year, we have inserted the sequences into a lentiviral vector to increase gene delivery efficiency.

Progress of our research:

During the third year, we have focused how to use the two peptides to modify lentiviral vectors, so that the generated lentiviral vectors are able to specifically target prostate cancer cells (Task 6).

We make progress following our SOW.

1. Insert sequences into lentiviral envelope protein gp41

As described in the annual report of last year, we have identified two peptides that can increase lentiviral infection of LNCaP prostate cells 2 to 4-fold. We hypothesize that by adding these two peptides to lentiviral envelope protein, we can make lentiviral vector to specifically infect LNCaP cells. As Figure 1 shown, gp41 protein is an envelope protein located on the surface of lentiviral vector. The N-terminus of this protein is extracellular. So it is possible to add our sequence to the N-terminus so that the added peptide sequences can interact with LNCaP surface protein. When the lentiviral vector attaches to the target cells, the other part of gp41 can induce the viral envelope to fuse with cell membrane and render the viral genome to enter the target cells.

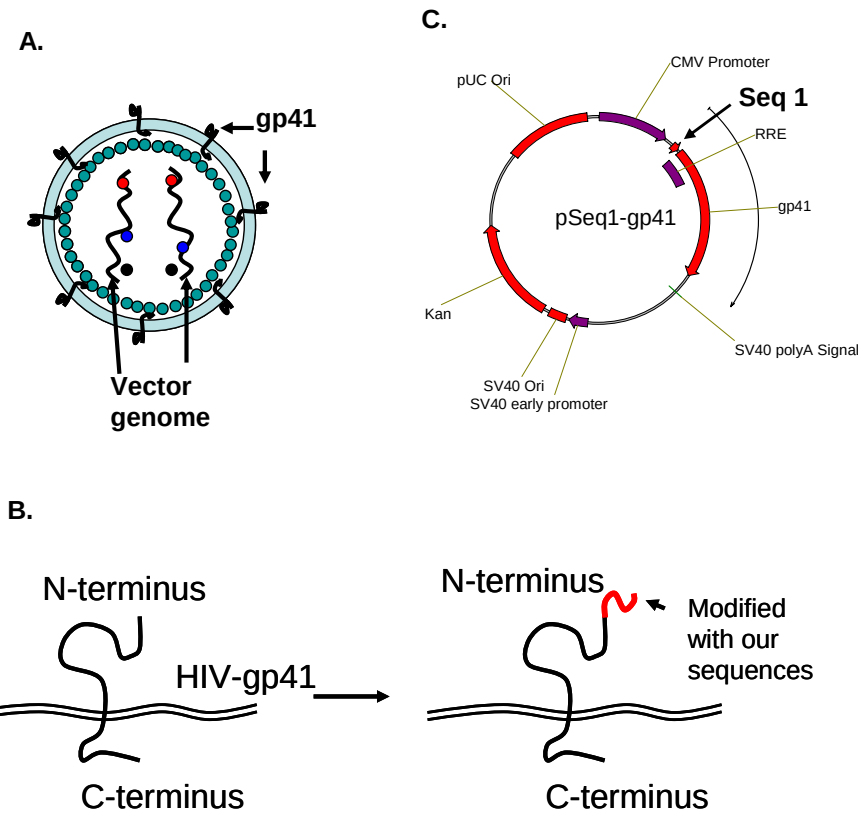

Fig. 1. Strategy of our approach to use two peptides with sequence of RGCICRCIGRGCICRCIG or YDSVLALSAALQAT R to modify lentiviral envelope protein gp41. A) A diagram of a lentiviral vector. B) Attachment of our peptide to gp41 at the N-terminus. C) The structure of a plasmid that carries the gp41 fusion fusion peptide.

2. Using modified lentiviral vectors to infect LNCaP cells

We used co-transfection method to generate lentiviral vectors that carry the enhanced green fluorescent protein (EGFP). The viral vector that have peptide

YDSVLALSAALQATR (P2) modified gp41 envelope protein was used to infect LNCaP cells in 24-well plates. The control vector that does not have gp41-P2 envelope protein on viral surface was also used to infect LNCaP cells as the control. Our results demonstrated that with the gp41-P2 envelope protein on the surface of the viral vector, lentiviral vectors increase infectivity by 30% to 70% as shown in Fig. 2.

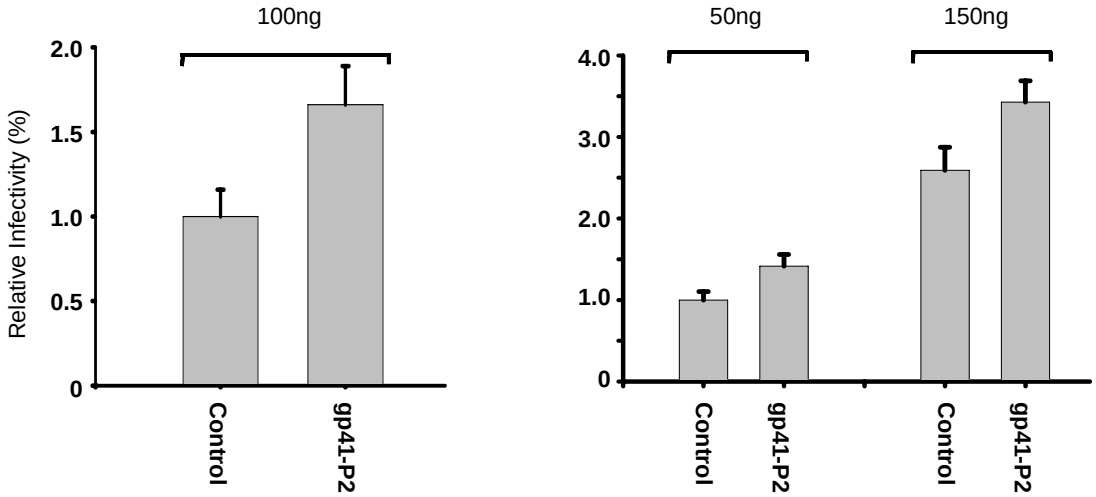

Fig. 2. Increased infectivity by peptide YDSVLALSAALQATR. LNCaP cells were infected by a EGFP-expression lentiviral vector with gp41-P2 envelope protein on viral surface or with no gp41-P2 (control) on viral surface. The left penal, Cells in 24-well plates were infected by viral vector of 100 ng p24 reading. The right penal, LNCaP cells infected by 50 and 150 ng of lentiviral

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Key Research accomplishments:

We have fused one of our peptides into a lentiviral envelope protein and found that the addition of this peptide increased gene delivery efficiency

Conclusions:

We have inserted two peptide sequences into a lentiviral surface protein gp41 and tested one. Our results demonstrated that with such sequence, gene delivery efficiency can be enhanced. However, the increase is not very high. It is expected by using different way to modify the lentiviral envelope gene with these two peptides, we can increase gene delivery efficient even further. We will insert multiple copies of our peptide sequences into gp41 and to insert these peptide sequences into other gp41 locations. We expect that by these approaches, we can further increase gene delivery efficiency.